GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2015), doi: 10.1111/gcbb.12303

Soil fungal and bacterial responses to conversion of open land to short-rotation woody biomass crops

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Abstract

Short-rotation woody biomass crops (SRWCs) have been proposed as an alternative feedstock for biofuel production in the northeastern US that leads to the conversion of current open land to woody plantations, potentially altering the soil microbial community structures and hence functions. We used pyrosequencing of 16S and 28S rRNA genes in soil to assess bacterial and fungal populations when 'marginal' grasslands were converted into willow (*Salix* spp.) and hybrid poplar (*Populus* spp.) plantations at two sites with similar soils and climate history in northern Michigan (Escanaba; ES) and Wisconsin (Rhinelander; RH). In only three growing seasons, the conversion significantly altered both the bacterial and fungal communities, which were most influenced by site and then vegetation. The fungal community showed greater change than the bacterial community in response to land conversion at both sites with substantial enrichment of putative pathogenic, ectomycorrhizal, and endophytic fungi associated with poplar and willow. Conversely, the bacterial community structures shifted, but to a lesser degree, with the new communities dissimilar at the two sites and most correlated with soil nutrient status. The bacterial phylum Nitrospirae increased after conversion and was negatively correlated to total soil nitrogen, but positively correlated to soil nitrate, and may be responsible for nitrate accumulation and the increased N_2O emissions previously reported following conversion at these sites. The legacy effect of a much longer grassland history and a second dry summer at the ES site may have influenced the grassland (control) microbial community to remain stable while it varied at the RH site.

Keywords: grassland, poplar, short-rotation woody biomass crop, soil bacterial community, soil fungal community, willow

Received 11 March 2015; revised version received 1 July 2015 and accepted 19 August 2015

Introduction

Biofuel production, including plant-derived bioethanol, biodiesel, and biogas, has the potential to significantly reduce global dependence on fossil fuels and provide a sustainable strategy to partially meet the growing energy demands (Demirbas, 2008; Ghatak, 2011). In 2005, 14.3% of the US corn harvest produced 1.48×10^{10} l of ethanol, and soybean oil extracted from 1.5% of the US soybean harvest produced 2.56×10^{8} l of biodiesel (Hill *et al.*, 2006). The US federal government adopted a goal to annually produce 36 billion gallons of alternative fuels by 2022 (EISA, 2007). To meet just half of this target, the production of corn-based bioethanol would require 65.8% of the 2005 corn harvest. The most likely outcome of increased production of corn-based ethanol would be higher food prices and a potential for food-related security crises. Short-rotation woody biomass crops (SRWCs) such as hybrid poplar (*Populus* spp.) and willow (*Salix* spp.) have been proposed as alternative options for biofuel feedstocks due to their fast growth and short harvest cycles (Labrecque & Teodorescu, 2005; Aylott *et al.*, 2008).

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The availability of limited arable area for food crop production is a major constraint for many countries, hence the proposal to use marginal lands for biofuel crops (Hill *et al*., 2006; Gopalakrishnan *et al*., 2009). Studies have indicated that marginal agricultural land can contribute to biomass production without impacting food production on prime cropland (Tang *et al*., 2010; Cai *et al*., 2011; Zhuang *et al*., 2011). In the northern Great Lake States (MI, WI and MN), widespread planting of hybrid poplar and willow on marginal open lands has been viewed as the best option for sustaining a regional bioenergy production system (Davis *et al*., 2012; Zalesny *et al*., 2012), and there is a long history of hybrid poplar breeding efforts in the region dating to the first energy crisis of the 1970s (Hansen, 1991). Open lands targeted for conversion consist of a mixture of marginal agricultural land, abandoned farms, hayfields, and pasturelands and make up a significant portion of the landscape matrix of this region (Mladenoff *et al*., 2015).

Soil microbial communities, particularly bacteria and fungi, have the potential to influence biofuel crop establishment on marginal lands through plant–microbe interactions that include nutrient acquisition, growth promotion, the alleviation of environmental stress, and disease bio-control (Glick, 1995; Lugtenberg & Kamilova, 2009; Compant *et al*., 2010). Plants also affect the soil microbial community (Loon, 2007; Bever *et al*., 2012) by shaping the diversity and composition through niche establishment, secretion of root exudates, symbiosis, and litter chemistry and directly altering soil structure, nutrient availability, and cycling (Yang & Crowley, 2000; Garbeva *et al*., 2004; Schweitzer *et al*., 2008; Thoms *et al*., 2010). Furthermore, microbial community structure is also largely driven by land use, vegetation, soil pH, and drainage (aeration) (Lauber *et al*., 2009; Rousk *et al*., 2010; Nacke *et al*., 2011; Hanson *et al*., 2012).

The establishment of SRWC plantations on marginal lands often entails conversion of existing grasslands to tree plantations. Previous studies at Escanaba (ES), Rhinelander, and other sites document that this conversion can have substantial impacts on soil nitrogen cycling and greenhouse gas emissions (Nikièma *et al*., 2012; Palmer *et al*., 2013). However, there is a knowledge gap concerning how such conversions, including new crop and tillage, affect soil microbial communities on marginal lands in the Great Lakes region, and the role that microbial community shifts may play in driving the observed increases in greenhouse gas emissions and other system functions.

To assess the impact of grassland to SRWC conversion, we examined the effects of converting hayfields to willow and hybrid poplar plantations on soil bacterial and fungal communities after three growing seasons at two sites in northern Michigan and Wisconsin through pyrosequencing of 16S and 28S rRNA genes in soil DNA. Our objectives were to determine the following: (i) the degree to which microbial communities shifted in response to land conversion, (ii) the degree to which community shifts were consistent between the two sites, and (iii) which taxa changed in this shift and the attributes of those taxa as they may infer functional changes in the plant–soil system.

Materials and methods

Field experiment design

The two field sites are located at Escanaba (ES), Michigan, USA (45°46′20″N, 87°11′43″W) and Rhinelander (RH), Wisconsin, USA (45°40′13″N, 89°12′45″W). The soil at the RH site is a loamy sand developed on well-sorted glacial outwash parent material, whereas the soil at ES is a fine sandy loam developed from limestone-derived glacial till. The RH site had been used for production of corn and other row crops until 2005, when it was converted to a cool-season grass hayfield. In contrast, the ES site was last cultivated in 1968, converted to cattle pasture in the 1970s, and had been used for hay production for at least the last 20 years. The 30-year mean annual precipitation at the ES site is 728 mm, while it is 675 mm at the RH site (Table S1). The experimental design is described in detail in Palmer *et al.* (2014). Briefly, identical experimental designs were initiated at both sites with 12 replicate plots randomly assigned to three treatments: (i) planted to willow, (ii) planted to hybrid poplar, or (iii) control plots, which were maintained in the prior land use (unplowed hayfield). Each plot was 40×40 m, with all samples collected from the inner 20×20 m to avoid edge effects and provide a buffer between plots.

In May 2010, all the existing vegetation was killed with glyphosate in the plots to be planted to poplar and willow, and then, these plots were cultivated with a moldboard plow and disked (Palmer *et al*., 2014). Hybrid poplar cultivar NM6 (*Populus maximowiczii* × *Populus nigra*) was planted at a standard density of 1900 stems ha^{-1}. Willow cultivar Fish Creek (*Salix purpurea*) was planted at a standard density of 14 000 stems ha^{-1}. No fertilizer was applied to any plot during this study. Control plots were left untouched as uncultivated mixed species grass hayfields, which represents the baseline, preconversion condition at each site.

Soil sampling and DNA extraction

In 2010, soil samples were collected immediately before conversion on May 11 at the ES site and on May 13 at the RH site. After three growing seasons, samples were collected on August 29, 2012, at the ES site and on September 17, 2012, at the RH site. Five bulk soil cores were randomly collected from each plot to a 10-cm depth (surface soil ~1 cm was removed before

 doi: 10.1111/gcbb.12303

sampling) using a soil probe. Soil from the cores was placed in plastic bags and kept on ice after collection and during transportation, and was stored at −20 °C in the laboratory until further processing. Five grams of soil from each core sample within the same plot was sieved (2-mm-mesh sieve), combined, and mixed thoroughly to make one sample to represent each plot. DNA was extracted from 0.5 g of the mixed soil sample using the Powersoil DNA Extraction kit (MOBIO Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions.

Amplification and 454 pyrosequencing

Primer sets 454–577f adapter-mid-AYTGGGYDTAAAGNG and 454–926r adapter-mid-CCGTCAATTCMTTTRAGT were used to amplify partial 16S rRNA genes, and primers LR3 mid-CCGTGTTTCAAGACGGG and LR0R mid-ACCCGCTGAACTTAAGC were used to amplify the 28S rRNA genes for all samples according to previously published protocols (Sul *et al*., 2011; Penton *et al*., 2013, respectively), where mid refers to a unique mid sequence used for sample sorting. The 28S rRNA amplicons were adapter ligated and bi-directionally sequenced on the 454 Life Sciences Titanium platform, using Lib-L kits (454 Life Sciences Corporation, Branford, CT, USA). All 16S rRNA samples were unidirectionally sequenced using Lib-A kits (454 Life Sciences Corporation). Sequencing was performed at Center for Integrated BioSystems, Utah State University, USA.

Measurement of soil characteristics

Soil characteristics, including pH, total nitrogen, ammonium, nitrate, total carbon, and C/N ratios, were determined on separate 10-cm core samples collected within 2 weeks of the initial and final sampling dates for soil microbial communities. Inorganic soil nitrate and ammonium concentrations were determined by extracting a 10-g subsample with 2 M potassium chloride for 1 h. Soil extracts were analyzed for ammonium and nitrate colorimetrically according to procedures described by Sinsabaugh *et al.* (2000) and Doane & Horwáth (2003), respectively. Total carbon, total nitrogen, and C/N ratios were determined on oven-dried soil via dry combustion on an elemental analyzer (Costech ECS 4010, Valencia, CA, USA). Soil pH was determined with a glass electrode in a 1 : 2 slurry of air-dried soil in 0.01 M $CaCl_2$.

Plant biomass

Leaf, stem, and fine-root biomass were determined at ES and RH sites for 2011 and 2012. Two 3.8 cm diameter × 10 cm deep soil cores were collected from each plot at 2–3 week intervals throughout the growing season. Fine roots (2 mm diameter) from these cores were hand sorted. Because there was significant within-plot variability that could potentially skew results with only two cores per plot per sample date, data were averaged across sampling times between June 1 and August 30 to calculate a growing-season average for each plot. Leaf and stem biomass production for poplar and willow plots were calculated by measuring tree heights and diameters, together with allometric biomass equations developed for our sites as described by Palmer *et al.* (2014). Because poplar and willow are deciduous species, we make the assumption that leaf biomass in a given year is a suitable proxy for leaf litterfall. Finally, aboveground herbaceous biomass from control plots was measured by collecting all live plant materials from a 0.37-m^2 sampling frame in mid-July of each year.

Sequence processing

Raw sequences were sorted according to barcode and processed through the RDP pyrosequencing pipeline (http://pyro.cme.msu.edu) with low quality (Q score <20) and short reads (length <200) removed. Chimeric reads were filtered using UChime (Edgar, 2010) followed by alignment and clustering at 97% and 95% nucleotide identity for 16S and 28S rRNA reads, respectively (Krüger *et al*., 2012; Shen *et al*., 2014). All the reads were classified using RDP naive Bayesian classifier (Wang *et al*., 2007). For all samples, the cluster data were randomly resampled to the sequence depth of the sample with lowest number of reads (2814 and 3637 reads for 16S and 28S rRNA genes, respectively) for downstream analysis. All sequences were deposited in the NCBI Sequence Read Archive (SRA) database (Accession numbers: SRX483129 and SRX483122).

Data analysis

Downstream analyses were performed in R (version 3.1.2; https://www.r-project.org) with package VEGAN (version 2.3-0; https://github.com/vegandevs/vegan) and PHEATMAP (1.0.2; https://cran.r-project.org/web/packages/pheatmap). Data were log or square root of relative abundance transformed to ensure normality and equal variance. Alpha-diversity (Shannon diversity and Chao richness) was calculated according to the re-sampled cluster data. Nonmetric multidimensional scaling (NMDS) was performed to illustrate the beta-diversity (Bray–Curtis distances) between treatments. Similarity percentage (SIMPER) analysis based on the estimation of average contribution of each individual microbial genus to the overall Bray–Curtis distances coupled with t-test was conducted to identify the indicator microbial groups between treatments (Warwick *et al*., 1990). Only the genera that showed significant difference ($P < 0.05$) between treatments were considered as indicators. Heatmap plots were generated to illustrate microbial community shifts between treatments. The data were log-transformed and sorted by the maximum value of each genus among all treatments at each site, of which the top 30 genera were used to generate heatmap plots. Zero was transformed to −6 instead of log-transformation, which was smaller than the lowest value after transformation for all samples. Variance partition analysis was conducted to estimate the proportion of the microbial community variance that was explained by environmental factors. Significant community differences were tested using analysis of similarity (ANOSIM) (Clarke, 1993). Mantel tests were performed to identify the correlation between microbial groups and environmental factors (Smouse *et al*., 1986). To determine the significance of differences, two-tailed, unpaired t-tests were

performed on individual microbial groups between treatments. One-way ANOVA followed by the Tukey's HSD test was performed to compare the means in R (version 3.1.2) with AGRICOLAE package (version 1.2-1; https://cran.r-project.org/web/packages/agricolae).

Results

General information from sequencing data

In total, 187 546 bacterial 16S and 281 888 fungal 28S rRNA gene reads passed quality trimming and chimera check with an average 5861 ± 2098 and 8809 ± 5041 reads per sample for 16S and 28S rRNA genes, respectively. After re-sampling, the average number of OTUs per sample was 1033 ± 113 and 1070 ± 209 for 16S and 28S rRNA, respectively. All 16S rRNA reads were classified into 23 phyla, 57 classes, and 504 genera with the average number of unclassified reads at 13.7 ± 4.0% at the phylum level, 18.6 ± 5.3% at the class level, and 33.5 ± 5.3% at the genus level. All 28S rRNA reads were classified into 8 phyla, 29 classes, and 538 genera with the average unclassified number of reads at 13.2 ± 7.5% at the phylum level, 18.8 ± 9.5% at the class level, and 39.4 ± 11.2% at the genus level. The top 10 most abundant bacterial classes across all samples in rank order were Alphaproteobacteria, Acidobacteria-Gp6, Gemmatimonadetes, Actinobacteria, Gammaproteobacteria, Acidobacteria-Gp4, Betaproteobacteria, Deltaproteobacteria, Acidobacteria-Gp3, and Planctomycetacia (Table S2). The top 10 most abundant fungal classes in rank order were Sordariomycetes, Agaricomycetes, Leotiomycetes, Dothideomycetes, Eurotiomycetes, Pezizomycetes, Chytridiomycetes, Tremellomycetes, Fungi incertae sedis, and Ustilaginomycetes (Table S3).

Patterns in microbial community compositions

The bacterial community at the ES site was compared between samples taken in May 2010 immediately prior to conversion and in August 2010 after the tree seedlings had begun to grow, but no differences were found in the communities (Fig. S1). As the trees were only seedlings and had no chance to impact the soil, further

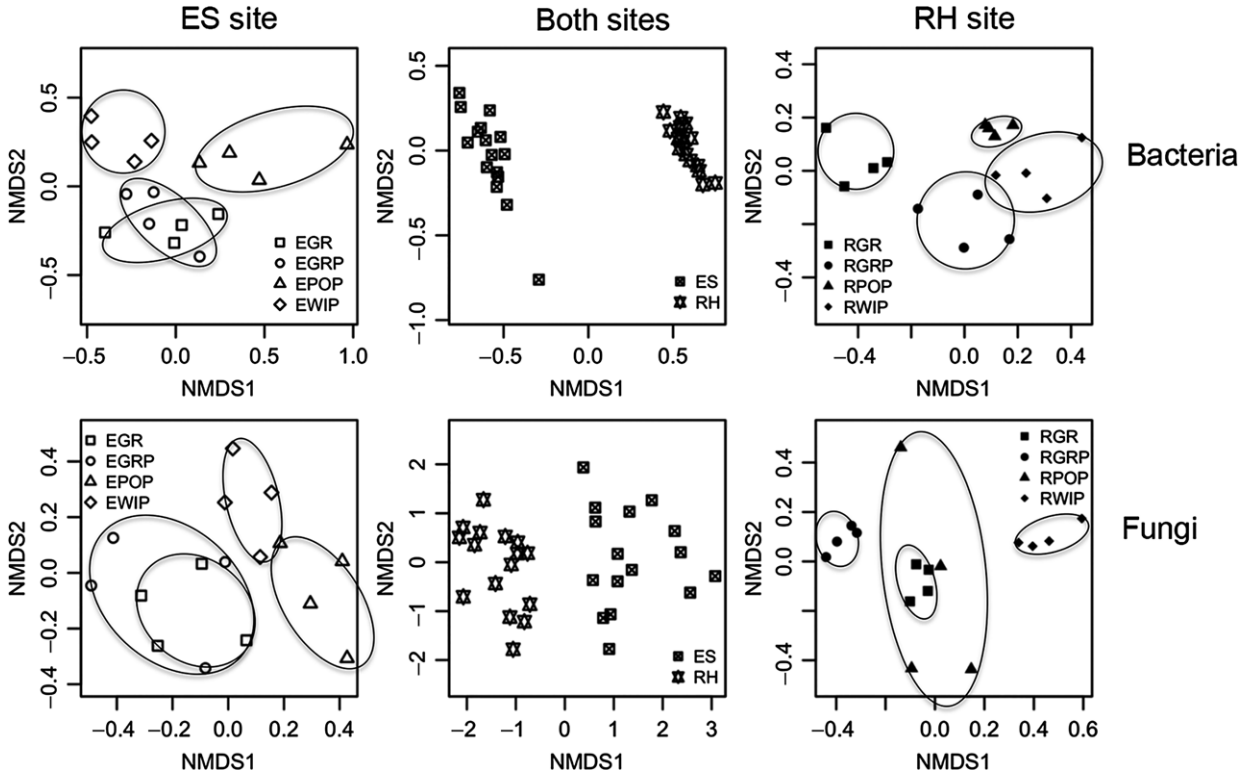

Fig. 1 Nonmetric multidimensional scaling (NMDS) ordination plots based on Bray–Curtis dissimilarity of Escanaba (ES) site samples, all samples, and Rhinelander (RH) site samples for bacteria and fungi. EGR and RGR are preconversion control plots at ES and RH sites, respectively. EGRP and RGRP are postconversion control plots at ES and RH sites, respectively. EPOP and RPOP are postconversion poplar plots at ES and RH sites, respectively. EWIP and RWIP are postconversion willow plots at ES and RH sites, respectively. ES represents all samples at Escanaba site, while RH represents all samples at Rhinelander site. NMDS stress values for bacterial communities are 0.079 (ES), 0.069 (both sites), and 0.105 (RH). NMDS stress values for fungal communities are 0.152 (ES), 0.148 (both sites), and 0.108 (RH).

 doi: 10.1111/gcbb.12303

sampling for community profiling was delayed until after the third season when the tree vegetation had more potential impact.

After three growing seasons postconversion, both the bacterial and fungal communities were altered at both sites (Fig. 1). All samples formed two groups based on location, and for each site, all samples could be further classified into three groups based on crop: grass (plots sampled at both pre- and postconversion times), willow, and poplar (Fig. 1). ANOSIM test revealed that converting grassland to willow and poplar significantly shifted both the bacterial and fungal communities into two distinct patterns based on vegetation (Table S4). Both bacterial and fungal community structures also differed in the RH control plots after three growing seasons, but not in the ES control plots (Table S4).

Conversion effect on microbial diversity

Changes in soil microbial diversities were influenced by both conversion and vegetation, but yielded different patterns at the two sites. At the ES site, converting grassland to poplar had no effect on fungal diversity, but it decreased bacterial diversity and richness significantly (Tukey's HSD test, $P < 0.05$) (Table 1). At the RH site, converting to poplar increased the bacterial diversity and richness (Tukey's HSD test, $P < 0.05$), while fungal diversity decreased after conversion (Tukey's HSD test, $P < 0.05$) (Table 1). In contrast, converting grassland to willow had no effect on bacterial diversity and richness with only a decrease in fungal diversity at the RH site (Tukey's HSD test, $P < 0.05$) (Table 1).

Conversion effect on microbial community composition

The effect of converting grassland to SRWCs on soil microbial composition was evaluated by comparing control plots with poplar and willow plots after the third growing season.

Poplar. Conversion altered both the bacterial and fungal communities from the genus to phylum levels. At the bacterial phylum level, the proportion of Acidobacteria increased at both sites after conversion (Tukey's HSD test, $P < 0.05$) with Proteobacteria, Planctomycetes, Gemmatimonadetes, and Actinobacteria as the most abundant bacterial phyla at both sites. After conversion, Sordariomycetes was the most abundant fungal class at both sites followed by Dothideomycetes, Agaricomycetes, Leotiomycetes, and Pezizomycetes. SIMPER analysis revealed that Acidobacteria groups, *Gemmatimonas*, and *Bradyrhizobium* were the most abundant bacterial contributing genera of conversion at both sites, with top 10 contributing genera comprising 58.9% and 52.6% of the total Bray–Curtis dissimilarity and 44.6% and 43.6% of the sequences at ES and RH site, respectively (Table S5). For fungi, only *Gibberella* was the shared indicator genus of conversion for the two sites. In the ES site poplar plots, *Hygrocybe* was the top contributing genus to the overall Bray–Curtis dissimilarity followed by *Schizothecium*, *Hebeloma*, and *Phaeodothis* with top 10 contributing genera comprising 56.4% of the total dissimilarity and 27.9% of the reads (Table S5). For RH, *Gaeumannomyces* was the top contributing genus to the dissimilarity followed by *Lycoperdon*, *Thelebolus*, and *Gibberella*, with top 10 contributing genera comprising 58.1% of the overall dissimilarity and 36.0% of the reads (Table S5).

Willow. Acidobacteria followed by Planctomycetes, Proteobacteria, Firmicutes, and Actinobacteria were the most abundant bacterial phyla in the ES site willow plots, while Proteobacteria followed by Acidobacteria, Actinobacteria, Bacteroidetes, and Gemmatimonadetes were the most abundant bacterial phyla at the RH site. Agaricomycetes was the most abundant fungal class at both sites followed by Sordariomycetes, Pezizomycetes, Leotiomycetes, and Dothideomycetes. For both sites, SIMPER analysis for this comparison was weighted heavily

Table 1 Measures of bacterial and fungal Chao richness and Shannon diversity for all treatments

			Chao richness		Shannon diversity	
Site	Crop	Conversion	Bacteria	Fungi	Bacteria	Fungi
Escanaba (ES)	Grass	Pre*	1748 ± 508^{b}	1389 ± 217^{ab}	6.51 ± 0.16^{ab}	6.13 ± 0.34^{abc}
ES	Grass	Post*	1707 ± 333^{b}	1568 ± 696^{ab}	6.48 ± 0.14^{abc}	6.04 ± 0.54^{abc}
ES	Poplar	Post	1201 ± 233^{c}	1464 ± 81^{ab}	6.25 ± 0.18^{d}	6.14 ± 0.25^{abc}
ES	Willow	Post	1516 ± 136^{bc}	1333 ± 354^{ab}	6.47 ± 0.09^{bc}	6.15 ± 0.47^{abc}
Rhinelander (RH)	Grass	Pre	1473 ± 158^{bc}	1541 ± 198^{ab}	6.44 ± 0.11^{bcd}	6.52 ± 0.15^{ab}
RH	Grass	Post	1444 ± 109^{bc}	1798 ± 108^{a}	6.31 ± 0.03^{cd}	6.68 ± 0.13^{a}
RH	Poplar	Post	2119 ± 69^{a}	1487 ± 384^{ab}	6.66 ± 0.07^{a}	6.02 ± 0.74^{bc}
RH	Willow	Post	1371 ± 114^{c}	1110 ± 233^{b}	6.26 ± 0.21^{d}	5.77 ± 0.35^{c}

*Pre and Post represent preconversion and postconversion, respectively.
Numbers in the same column with the same letter are not significantly different (Tukey's HSD test, $P > 0.05$, $n = 4$).

 doi: 10.1111/gcbb.12303

Table 2 Relative abundance of arbuscular mycorrhizal fungi genera detected in all treatments

Site	Crop	Conversion	Entrophospora (%)	Pacispora (%)	Glomus (%)	Paraglomus (%)
Escanaba (ES)	Grass	Pre*	ND	ND	0.01 ± 0.02b	0.01 ± 0.02a
ES	Grass	Post*	ND	ND	0.31 ± 0.28a	ND
ES	Poplar	Post	0 ± 0.01a	0.01 ± 0.01a	0.07 ± 0.12ab	0.02 ± 0.02a
ES	Willow	Post	ND	0.06 ± 0.12a	0.13 ± 0.17ab	0.02 ± 0.03a
Rhinelander (RH)	Grass	Pre	0.01 ± 0.01a	ND	0.02 ± 0.04ab	ND
RH	Grass	Post	ND	0.01 ± 0.01a	0.04 ± 0.07ab	0 ± 0.01a
RH	Poplar	Post	ND	ND	0.01 ± 0.01b	ND
RH	Willow	Post	0.01 ± 0.01a	ND	0.01 ± 0.01b	ND

*Pre and Post represent preconversion and postconversion, respectively.
Numbers in the same column with the same letter are not significantly different (Tukey's HSD test, $P > 0.05$, $n = 4$). ND means not detected. '0' is <0.005.

toward a few bacterial genera such as *Acidobacteria* groups, *Gemmatimonas*, and *Bradyrhizobium* with top 10 contributing genera comprising 53.0% and 54.7% of the total Bray–Curtis dissimilarity and 43.6% and 48.4% of the sequences at ES and RH sites, respectively (Table S6). For fungi, at the ES site, *Hygrocybe* contributed most (13.2%) to the Bray–Curtis dissimilarity followed by *Hebeloma*, *Sorocybe*, and *Clavulinopsis* with top 10 contributing genera comprising 60.9% of the overall dissimilarity and 26.7% of the reads. At the RH site *Hebeloma* contributed most (16.4%) to the Bray–Curtis dissimilarity followed by *Minimedusa*, *Thelebolus*, and *Peziza* with top 10 contributing genera comprising 63.1% of the overall dissimilarity and 36.9% of the reads (Table S6).

Arbuscular mycorrhizal fungi. In total, 1.3% of the total reads per sample were classified as arbuscular mycorrhizal fungi (AMF). Four AMF genera including *Entrophospora*, *Pacispora*, *Glomus*, and *Paraglomus* were detected, of which *Glomus* was consistently detected in all treatments and was the most dominant AMF genus (Table 2). Compared to the RH site, more AMF genera were detected in SRWC plots at the ES site. At both sites, the relative abundance of *Glomus* increased in control plots after three growing seasons (Table 2). Compared to control plots, the relative abundance of *Glomus* is lower in SRWC plots, though the difference is insignificant (Table 2).

Comparisons between bacteria and fungi. At both sites, conversion to SRWCs led to alterations in fungal composition with transitions occurring in both the rare (relative abundance <0.01%) and dominant populations (relative abundance >1%), for example, fungal groups such as *Hygrocybe*, *Hebeloma*, *Geopora*, *Cortinariaceae*, and *Inocybe*. In contrast, there was only a slight though significant variation in bacterial composition with no apparent shift between rare and dominant bacterial groups (Fig. 2). Furthermore, the majority of bacterial indicator genera such as *Bradyrhizobium*, *Gemmatimonas*, and *Nitrobacter* showed contrasting patterns at the two sites, while most of the fungal indicators such as *Hebeloma*, *Schizothecium*, *Gibberella*, *Geopora*, and *Peziza* exhibited the same shift at the two sites (Tables S5 and S6, Fig. 2).

Biomass

Measured or estimated leaf, stem, and fine-root biomass are presented in Table 3. For both sites, the control plots had the highest leaf biomass followed by willow and poplar in 2011 (Tukey's HSD test, $P < 0.05$) and 2012 (Tukey's HSD test, $P < 0.05$). Due to decreased leaf biomass in control plot (ES: Tukey's HSD test, $P > 0.05$; RH: Tukey's HSD test, $P < 0.05$) and a nonsignificant increase of leaf biomass in poplar and willow plots (Tukey's HSD test, $P > 0.05$) in 2012, the difference in leaf biomass between control and willow plots was insignificant. In addition, at the ES site, no significant difference of leaf biomass between poplar and willow was observed (Tukey's HSD test, $P > 0.05$), while willow had significantly higher leaf biomass than poplar in 2011 and 2012 at the RH site

Fig. 2 Heatmap displaying the relative abundances of top 30 bacterial and fungal genera for all treatments. The key from white to black represents the least abundant to most abundant, and the numbers represent log-transformed relative abundances of the microbial community. EGR and RGR are preconversion control plots at Escanaba (ES) and Rhinelander (RH) sites, respectively. EGRP and RGRP are postconversion control plots at ES and RH sites, respectively. EPOP and RPOP are postconversion poplar plots at ES and RH sites, respectively. EWIP and RWIP are postconversion willow plots at ES and RH sites, respectively.

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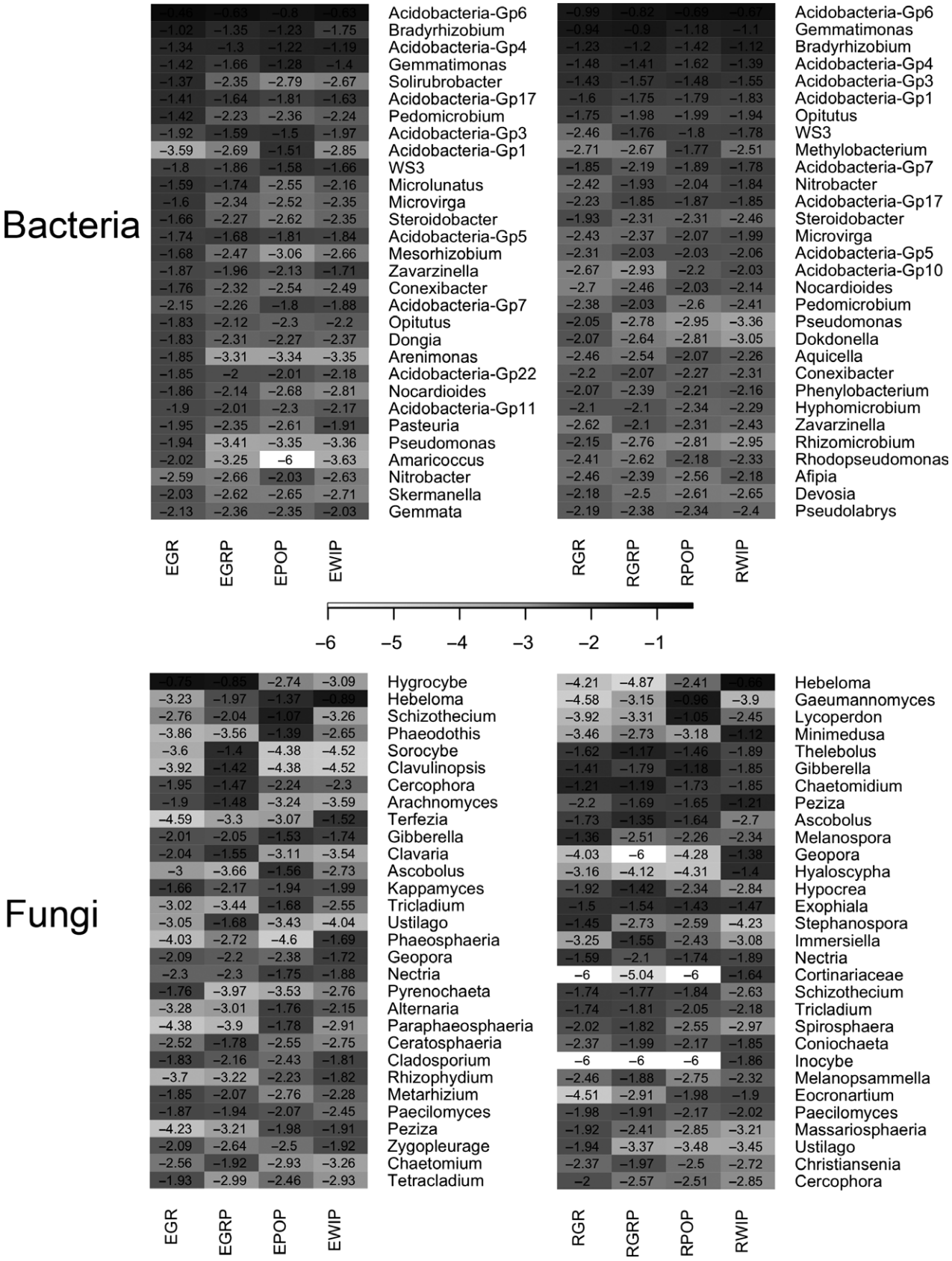

Table 3 Biomass of leaf, stem, and fine roots in 2011 and 2012 at Escanaba (ES) and Rhinelander (RH) sites

		Leaf		Stem		Root	
Site	Crop	2011	2012	2011	2012	2011	2012
ES	Grass	1.55 ± 0.38b	1.48 ± 0.35b	NA	NA	4.22 ± 0.75b	5.92 ± 0.93b
ES	Poplar	0.12 ± 0.06c	0.18 ± 0.1c	0.47 ± 0.32b	0.81 ± 0.51bc	0.79 ± 0.18c	1.28 ± 0.68c
ES	Willow	0.52 ± 0.33c	0.68 ± 0.34c	1.51 ± 0.98b	2.81 ± 1.7b	0.97 ± 0.50c	1.97 ± 0.77c
RH	Grass	3.49 ± 0.56a	2.45 ± 0.28a	NA	NA	10.09 ± 2.51a	9.00 ± 2.35a
RH	Poplar	0.19 ± 0.05c	0.54 ± 0.14c	0.83 ± 0.3b	3.29 ± 1b	2.42 ± 0.28bc	2.54 ± 0.70c
RH	Willow	2.15 ± 0.73b	2.25 ± 0.51a	5.68 ± 1.33a	10.67 ± 1.83a	1.53 ± 1.29bc	2.93 ± 1.00c

Numbers in the same column with the same letter are not significantly different (Tukey's HSD test, $P > 0.05$, $n = 4$). NA means data not available. Leaf and stem biomass for willow and poplar were calculated from tree diameter and height measurements as described by Palmer *et al.* (2014), whereas grass biomass in control plots was directly measured. The 2012 grass leaf data may underrepresent the yield because it is from an adjacent area as the control plot was inadvertently mowed.

(Tukey's HSD test, $P < 0.05$). Willow had significantly higher stem biomass than poplar at both sites in both years (Tukey's HSD test, $P < 0.05$) except for the ES site in 2011. As found for the leaf biomass, the control plots had the highest fine-root biomass followed by willow and poplar at both sites in both years (Tukey's HSD test, $P < 0.05$), while no significant differences between willow and poplar were observed at both sites in both years (Tukey's HSD test, $P > 0.05$). In addition, higher fine-root biomass was observed in the drier year (2011 for the RH site and 2012 for the ES site) in control plots at both sites, while the willow and poplar fine-roots biomass was higher in 2012 at both sites; however, the differences were insignificant (Tukey's HSD test, $P > 0.05$).

Soil characteristics and microbial community variation

Soil pH ranged from 5.18 to 5.54, except the preconversion ES site (6.14), which was significantly higher (Tukey's HSD test, $P < 0.05$) (Table 4). Before conversion, the ES site had higher total nitrogen than the RH site (Tukey's HSD test, $P < 0.05$) though total nitrogen did not differ after conversion. Compared to the control plots, conversion to SRWCs only altered the total nitrogen in the willow plots at the ES site for which total nitrogen decreased 18% (Tukey's HSD test, $P < 0.05$). Nitrate did not change in control plots at the two sites over the three growing seasons. Conversion to SRWCs significantly increased nitrate from 3.2- to 11.6-fold (Tukey's HSD test, $P < 0.05$), except in the RH willow plots, for which the average is a nonsignificant 1.4-fold increase over the control plots. Before conversion, the ES site had higher total carbon than the RH site. After three growing seasons, total carbon decreased from 24.4 to 19.8 mg g^{-1} in the ES control plots (Tukey's HSD test, $P < 0.05$), while it increased in the control plots at the RH site from 18.6 to 22.1 mg g^{-1} (Tukey's HSD test, $P < 0.05$). Compared to the control plots, converting to SRWCs did not influence total carbon at the RH site. However, total carbon decreased significantly at the ES site after conversion to SRWCs (Tukey's HSD test, $P < 0.05$). At the RH site, C/N was higher before conversion and after three growing seasons, and C/N dropped significantly in the control plots at both sites (Tukey's HSD test, $P < 0.05$). Conversion to SRWCs did not change the soil C/N, except in willow plots at the ES site, in which the C/N decreased (Tukey's HSD test, $P < 0.05$).

Crop type, year (includes climate differences), and soil characteristics were evaluated for their contribution to microbial community variation. These factors explained ~59% of the community variation (Table 5). Crop type was identified as the main factor that influenced both the bacterial and fungal community shifts (Table 5). Only for the RH site did the bacterial community exhibit a significant correlation with time ($r = 0.67$, $P = 0.002$), agreeing with the previous ordinations. Total nitrogen had strong correlation with bacterial community structure (ES: $r = 0.31$, $P = 0.014$; RH: $r = 0.37$, $P = 0.007$), but not with fungal community structure. Nitrate ($r = 0.24$, $P = 0.048$) and ammonium ($r = 0.25$, $P = 0.026$) were correlated with bacterial community structures at the ES site, but not at the RH site. Total carbon ($r = 0.24$, $P = 0.024$) and C/N ($r = 0.31$, $P = 0.019$) exhibited a correlation with the bacterial community structure at the ES and RH sites, respectively. Soil pH did not show any correlation with microbial community structure, probably because differences were minor. The bacterial phylum Nitrospirae significantly increased in SRWC plots (Turkey's HSD test, $P < 0.05$). Its relative abundance was negatively correlated to soil total nitrogen ($r = -0.48$, $P = 0.005$), but positively correlated to soil nitrate ($r = 0.4$, $P = 0.023$).

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Table 4 Soil characteristics of all treatments at Escanaba (ES) and Rhinelander (RH) sites

Site	Crop	Conversion	C/N (mass/mass)	Total C (%)	Total N (%)	pH	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})
ES	Grass	Pre*	12.94 ± 0.69^{b}	2.44 ± 0.08^{a}	0.19 ± 0.01^{a}	6.14 ± 0.92^{a}	1.19 ± 0.36^{c}	0.19 ± 0.03^{c}
ES	Grass	Post*	11.41 ± 1.30^{c}	1.98 ± 0.29^{bc}	0.17 ± 0.01^{ab}	5.18 ± 0.34^{b}	1.4 ± 0.28^{bc}	0.20 ± 0.20^{c}
ES	Poplar	Post	10.43 ± 1.00^{cd}	1.59 ± 0.26^{de}	0.15 ± 0.02^{bc}	5.43 ± 0.22^{b}	0.77 ± 0.31^{c}	2.32 ± 1.20^{a}
ES	Willow	Post	10.19 ± 0.29^{d}	1.40 ± 0.24^{e}	0.14 ± 0.02^{cd}	5.18 ± 0.15^{b}	1.07 ± 0.40^{c}	1.42 ± 1.08^{ab}
RH	Grass	Pre	14.77 ± 0.79^{a}	1.86 ± 0.17^{cd}	0.13 ± 0.02^{d}	5.37 ± 0.32^{b}	1.03 ± 0.19^{c}	0.27 ± 0.29^{c}
RH	Grass	Post	12.84 ± 0.47^{b}	2.21 ± 0.26^{ab}	0.17 ± 0.01^{ab}	5.47 ± 0.08^{b}	2.35 ± 0.88^{ab}	0.55 ± 0.13^{bc}
RH	Poplar	Post	12.56 ± 0.59^{b}	1.92 ± 0.15^{bc}	0.15 ± 0.01^{bc}	5.54 ± 0.13^{b}	1.52 ± 0.47^{bc}	1.77 ± 0.68^{a}
RH	Willow	Post	13.36 ± 0.56^{b}	2.11 ± 0.28^{bc}	0.16 ± 0.02^{bc}	5.38 ± 0.05^{b}	2.64 ± 1.62^{a}	0.76 ± 0.12^{bc}

*Pre and Post represent preconversion and postconversion, respectively.
Numbers in the same column with the same letter are not significantly different (Tukey's HSD test, $P > 0.05$, $n = 4$).

Table 5 Variance partition analysis and mantel test between microbial community and environmental factors

	Escanaba site						Rhinelander site					
	Bacteria			Fungi			Bacteria			Fungi		
Factor	Explained* (%)	*r*†	*P*†	Explained (%)	*r*	*P*	Explained (%)	*r*	*P*	Explained (%)	*r*	*P*
Crop	12.79	**0.43**	0.002	16.14	**0.29**	0.005	13.72	**0.39**	0.006	21.71	**0.55**	0.001
Year	5.40	0.07	0.282	6.60	0.04	0.353	6.38	**0.67**	0.002	6.86	−0.08	0.721
Total N	6.21	**0.31**	0.014	7.05	0.02	0.407	6.12	**0.37**	0.007	6.18	−0.17	0.922
NO_3^-	6.42	**0.24**	0.048	8.24	0.11	0.159	6.06	0.02	0.429	5.16	−0.07	0.647
NH_4^+	6.27	**0.25**	0.026	8.93	**0.33**	0.002	6.20	−0.02	0.502	4.19	−0.06	0.646
Total C	6.06	**0.24**	0.024	6.92	0.06	0.275	6.16	0.04	0.314	6.21	−0.01	0.51
C/N	6.29	0.08	0.230	6.70	0.13	0.103	6.17	**0.31**	0.019	6.02	0.01	0.477
pH	5.74	−0.05	0.592	3.91	0.01	0.402	6.14	0.05	0.323	3.21	−0.26	0.954

*Proportion of variance could be explained returned by variance partition analysis.
†*R* and *P* values returned by mantel test. *R*-value in bold means significantly correlated ($P < 0.05$, $n = 16$).

Discussion

Microbial community and environmental factors

Although the two experimental sites were relatively close to each other (~200 km apart, nearly identical latitude) with similar climate, soil type, and forested history during soil development and had identical experimental designs, the inherent site characteristics led to, in some cases, opposite shifts in patterns of soil microbial diversity and composition. These differences are likely due to the different compositions of the initial soil microbial communities that may have fundamentally influenced the different shift patterns observed at the two sites (Wakelin *et al*., 2008). There is also a difference in soil nutrients; ammonium and nitrate were significantly correlated to the microbial community structure at the ES site, but not at the RH site.

Although site differences were a factor, crop type was the main factor that influenced microbial community composition at each site. This agreed with previous studies on microbial communities in bulk and rhizosphere soils of three typical *Verticillium* host plants (Smalla *et al*., 2001), in forests in Britain (Grayston & Prescott, 2005), France (Lejon *et al*., 2005), and Malaysia (Ushio *et al*., 2008) and in a pasture to woody biomass conversion in Brazil (Rachid *et al*., 2015). This may be due to allelopathy, different chemical resources (e.g., root exudates) provided by the new roots and litter, and host selection for symbionts (Broeckling *et al*., 2008; Turner *et al*., 2013). In this study, roots more so than litter likely provided the new carbon for microbial growth as the soil core sampled the top 10 cm soil with the surface soil (~1 cm) removed and the litter impact was only at the surface and from the 2011 season as leaves had not fallen at the time of our 2012 sampling.

Soil microbial community structure can also be responsive to seasonality or larger climate differences (Cruz-Martínez *et al*., 2009; Andersson *et al*., 2010). In our case, both sites experienced wetter than normal growing seasons in the planting year of 2010 and drier than normal growing seasons in 2011. In 2012, RH rebounded to near normal precipitation, whereas ES

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was even drier (Table S1), which severely restricted stem and root growth of both willow and poplar despite more fertile soil at ES (Table 3). The effect of this second consecutive dry season at ES is consistent with significant variation being observed only at the RH site for both bacteria and fungi.

Historically, soil and vegetation at the ES site had remained undisturbed for 42 years before the start of our experiment. After four decades of adaptation to these conditions, the microbial community at the ES site was likely more resilient, thus possibly explaining the absence of temporal variation in control plots at this site. In contrast, the RH was under active cultivation until only 5 years prior to the start of the experiment. Thus, the significant temporal variation in control plots at this site suggests that the soil microbial community was still responding to the earlier conversion from row crops to grass. In support of this finding, a previous study reported that land-use history had a stronger impact on soil microbial community composition than aboveground vegetation and soil properties (Jangid *et al*., 2011). Thus, the combination of land-use history and climate prior to the initiation of the experiment may have impacted the temporal variability among these sites.

Conversion effect on soil microbial compositions

In the following discussion, we comment on potential function(s) of identified bacterial and fungal groups based on information from group members that have been studied. However, the extent to which these traits reflect the function of the whole group will vary with trait, group, and resolution of the marker gene, and as such should be considered only as potential ecological roles.

Conversion of grassland to SRWCs resulted in microbial community shifts at both sites. This was discernable by the genera associated with grasses and SRWC trees that were identified as contributing to the majority of the dissimilarity by SIMPER. The bacterial indicator genera were similar for all treatments at the two experimental sites. Acidobacteria groups, the most abundant bacterial groups in SRWCs plots, were the main indicators for postconversion. Acidobacteria are widespread in nature and have been reported as the most abundant bacterial group in poplar and willow rhizospheres (De Cárcer *et al*., 2007; Gottel *et al*., 2011). Other shared top indicator genera, such as *Gemmatimonas*, *Nocardioides*, and *Bradyrhizobium* are also widespread. *Gemmatimonas*, specifically *G. aurantiaca*, was reported as a polyphosphate-accumulating bacterium that could be used for biological phosphorus removal (Zhang, 2003). *Nocardioides* was reported to accumulate in soil after poplar was planted (Hur *et al*., 2011) and has been described as an endophytic bacteria in poplar (Ulrich *et al*., 2008). *Nocardioides* has also been reported as enriched in soils with persistent organic pollutants (Golovleva *et al*., 1990; Iwabuchi & Harayama, 1997; Cho *et al*., 2000), which suggests that it may have the capacity to degrade complex organic compounds, such as those found in tree litter. *Bradyrhizobium* is a well-known N_2-fixing bacterial genus, reported as one of the dominant bacterial groups in the rhizo- and endosphere of poplar (Gottel *et al*., 2011). However, these indicator genera did not show consistent shift patterns at both sites after conversion. Deforestation was reported to alter soil microbial populations from *Fibrobacter* and *Syntrophomonas* assemblages in forest soil to *Burkholderia* and *Rhizobium–Agrobacterium* assemblages when the soil was converted to pasture (Nusslein & Tiedje, 1999). In our study, *Burkholderia* and *Rhizobium* both decreased in abundance after conversion and *Fibrobacter* and *Syntrophomonas* were not detected in any of the plots. These results indicate that *Burkholderia* and *Rhizobium* might be more dominant in grassland. Lastly, the bacterial phylum Nitrospirae was found significantly increased in poplar and willow plots after conversion, which is negatively correlated to soil total nitrogen ($r = -0.48$, $P < 0.05$) and positively correlated to nitrate ($r = 0.4$, $P < 0.05$). One confounding factor in these analyses involves the temporal scale and the resulting comparisons between SRWC plots to the control. As there was no sprayed and plowed control (vs. the native grassland control), there is the potential for changes in microbial community composition due to these pretreatments. However, the majority of community changes after 3 years appear to be most related to vegetation type and the direct comparisons between SRWC's remain valid, although a portion of the shared microbial community between SRWC's may be have been shaped by the initial spray/plow treatment.

For fungi, the grassland contributing genera reflect the characteristics of the site. At the ES site, *Hygrocybe* is a leading contributor, the dominant fungal group in grass plots. These gilled fungi are associates of mosses and are characteristic of old, unimproved grasslands (Rotheroe *et al*., 1996). Another contributor of the grassland at ES site was the genus *Clavulinopsis*. This genus belongs to the Clavariaceae, described as part of the CHEG (Clavariaceae, Hygrocybe, Entolomataceae, and Geoglossaceae) community that have been associated with old, undisturbed, unimproved grasslands (Russell, 2005; Moore *et al*., 2008). As a likely endophyte of the native grasses, *Cercophora* (specifically *coprophila*) has been identified as an endophyte of *Ammophila arenaria*, a coastal grass (Sánchez Márquez *et al*., 2008), and *Stipa grandis* (needlegrass) (Su *et al*., 2010). Species within the

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genus *Clavaria* have close physiological relationships to roots that possess *Ericoid* mycorrhizae (Englander & Hull, 1980; Petersen & Litten, 1989). Members of the genus *Ustilago* are smut fungi that are parasitic on grasses (Kirk *et al*., 2008).

At the RH site, *Trichoderma* was a contributing genus. As the anamorph of *Hypocrea*, the species *hamatum*, *harzianum*, and *koningii* have been isolated from roots of wheat and rye grass (Dewan & Sivasithamparam, 1988) and thus may be associated with grasses present at this site. Interestingly, some *Trichoderma* species have been found to inhibit root rot by *Gaeumannomyces graminis var*. *tritici*, which was found in reduced abundance in the control plot at this site. *Chaetosphaeria* have been isolated as endophytes of sand couch grass (*Elymus farctus*) (Sánchez Márquez *et al*., 2008). Little is known of *Thelebolus* except that it has been isolated as an endophyte of *Scandinavian* small reed (*C*. *phragmitoides*).

Among the top 20 fungal genera, *Hebeloma*, *Gibberella*, and *Peziza* showed a consistent shift after conversion to either poplar or willow at both sites. *Basidiomycota*, associated with high-lignin litter degradation (Blackwood *et al*., 2007) and very common in forest soils, were higher in abundance in the SRWC plots. Lignin degradation-associated fungi belonging to the genera *Hebeloma* and *Peziza* have been identified on rotted alder, willow, and poplar (Beug *et al*., 2014). These all increased abundance in SRWC plots at both sites, and *Hebeloma* was the overall most abundant Basidiomycota group in SRWC plots. *Hebeloma* (sp. *fastibile*) and *Peziza* (e.g., *Peziza ostrcoderma*) have also been observed to form ectomycorrhiza with Balsam poplar (*Populus balsamea*; Siemens & Zwiazek, 2008) and *Salix* (willow) (Baum *et al*., 2009; Danielsen *et al*., 2012). *Gibberella* (telemorph *Fusarium*), a potential pathogenic lineage in poplar, has been found in high abundance of diseased willow plants and during first year growth in previously arable land (Corredor *et al*., 2012). In this study, *Gibberella* increased four times in poplar plots and two times in willow plots after conversion. However, not all species are pathogenic and this lineage is poorly resolved with the 28S rRNA gene.

Differences between bacteria and fungi

Conversion significantly altered fungal compositions leading to transitions between rare fungal groups and the dominant ones. However, conversion did not cause a dramatic change in the dominant bacterial genera and no apparent shift between rare and dominant bacterial groups. In addition, fungal communities had more consistent shift patterns at the two sites, indicating that fungi appear more responsive to conversion and the community is more specific to the aboveground vegetation than the bacterial community. This is in agreement with a previous study on afforestation of pastures with *Pinus radiate* (Macdonald *et al*., 2009) where the soil fungal community was more affected by land conversion than the bacterial community. In addition, Tedersoo *et al*. (2014) found large differences in fungal communities between forested and treeless ecosystems and that fungal diversity was linked to plant diversity. Agricultural tillage is also known to negatively affect fungi more than bacteria (Stahl *et al*., 1999; Kubicek & Druzhinina, 2007), but those studies are with repeated tillage, not the one time tillage three growing seasons earlier as was used here. Nonetheless, the early tillage event was a major disturbance and would have allowed some populations to gain an early advantage, which may have persisted, especially if those populations were effective competitors for tree leaf litter or root carbon. AMF is widely acknowledged as beneficial fungal group for plants (Bainard *et al*., 2012a). In this study, four AMF genera were detected and only *Glomus* was consistently detected in both grass and SRWC plots. The failure of detection of other AMF groups is likely due to (i) the use of a universal fungal primer in this study, not an AMF specific primer set, and/or (ii) low numbers of non-*Glomus* AMF groups in agroecosystems (Bainard *et al*., 2012b). As such, deeper sequencing may be required to detect these rare AMF groups. *Glomus* was in higher relative abundance in the dry year than the wetter year in control plots at both sites, in agreement with previous studies that AMF would benefit the plant in water uptake in drought (Doubková *et al*., 2013; Jayne & Quigley, 2014). Overall, SRWC conversion decreased the relative abundance of *Glomus*, compared to the control. Conversely, some ectomycorrhizal fungi (ECM), namely Pezizales, Hebeloma, Cortinariaceae, Ceratobasidiaceae, Cantharellales, Atheliales, Tricholomataceae, and Thelephorales, increased in SRWC plots, though others decreased (Table S7). Overall, more ECM fungi increased in relative abundance in SRWC plots. Hence, there was the expected switch from AM fungi to ECM fungi with change in vegetation, which is important to tree productivity on these marginal lands (Smith & Read, 2010; Zuccaro *et al*., 2014).

Soil microbial community and soil nitrogen

If nitrate is present in excess of plant demand, there is potential for leaching of nitrate and N_2O emissions (Galloway *et al*., 2003). In our previous study at these sites (Palmer *et al*., 2013), we observed a decrease in ammonium and large spikes in soil extractable NO_3^-, NO_3^-

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leaching losses, and soil N_2O emissions in the first 2 years following conversion from grassland to poplar or willow plantations. This trend is consistent with a decrease in ammonium due to plant uptake and nitrification, the latter resulting in nitrate accumulation and N_2O production. The bacterial phylum Nitrospirae, a well-known nitrite-oxidizing bacterial group, significantly increased in abundance in the SRWC plots. Glyphosate application alone, associated with the SRWC conversion, has been shown to reduce Nitrospirae abundance (Barriuso *et al*., 2011), but in our case, the tillage would have enhanced N mineralization providing substrate for their growth and producing nitrate. Their increase was negatively correlated to soil total nitrogen, but positively correlated to soil nitrate, which is consistent with our previous findings on increased N_2O emissions at the SRWC sites (Palmer *et al*., 2013).

Acknowledgements

This project was funded by the US Department of Energy (DOE) Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494 and DOE OBP Office of Energy Efficiency and Renewable Energy DE-AC05-76RL01830) to Michigan State University and University of Wisconsin-Madison, by US Department of Energy award # DE-EE-0000280, and by the US Department of Agriculture, Agriculture and Food Research Initiative, Sustainable Bioenergy Program (2010-03866).

We thank Michael Cook, Christopher Johnson, Marin Palmer, and Paul Irving for assistance with the fieldwork and soil analyses, and Eliane Aparecida Gomes, Mark Charbonneau, Adam Dingens, and Julian Yu for help with the DNA-based work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Nonmetric Multidimensional Scaling (NMDS) ordination plots based on Bray–Curtis dissimilarity of RH site samples for bacteria.
Table S1. Annual and growing season precipitation at Escanaba and Rhinelander sites.
Table S2. Top 10 abundant bacterial and fungal classes of preconversion control (EGR), postconversion control (EGRP), poplar (EPOP), and willow (EWIP) plots at ES site.
Table S3. Top 10 abundant bacterial and fungal classes of preconversion control (EGR), postconversion control (EGRP), poplar (EPOP), and willow (EWIP) plots at RH site.
Table S4. F and *P* values returned by ANOSIM test of pairwise comparisons.
Table S5. Similarity percentage (SIMPER) analysis showing the top contributing bacterial and fungal genera to the difference between poplar and control plots at ES and RH sites.
Table S6. Similarity percentage (SIMPER) analysis showing the top contributing bacterial and fungal genera to the difference between willow and control plots at ES and RH sites.
Table S7. Relative abundance of ectomycorrhiza (ECM) fungi in all treatments.

